

CLAIMS

We claim:

1. A metabolic profiling method for identifying a metabolic state of a subject biological
5 sample, wherein said method comprises analyzing in an automated pattern recognition system
data obtained from the subject biological sample by a spectroscopic or chromatographic
technique in comparison to data obtained from a plurality of other known biological samples
by the spectroscopic or chromatographic technique to determine a comparable metabolic
state, wherein the biological samples are obtained from organisms grown under controlled
10 conditions, and wherein the data is a compilation of a plurality of observed metabolites.
2. The method of claim 1, wherein the chromatographic technique is gas
chromatography.
- 15 3. The method of claim 1, wherein the spectroscopic technique is nuclear magnetic
resonance spectroscopy.
4. The method of claim 1, wherein the spectroscopic technique is mass spectroscopy.
- 20 5. The method of claim 1, wherein said method employs data obtained from both
chromatographic and spectroscopic techniques.
6. The method of claim 1, wherein the pattern recognition analysis system comprises a
neural network analysis.
- 25 7. The method of claim 1, wherein the metabolic state is selected from the group
consisting of:
 - a. inhibition of acetyl CoA carboxylase (ACCase);
 - b. inhibition of acetolactate synthase (ALS) or acetohydroxyacid synthase
30 (AHAS);
 - c. inhibition of photosynthesis at photosystem II;
 - d. photosystem-I-electron diversion;
 - e. inhibition of protoporphyrinogen oxidase (PPO);

- f. inhibition of carotenoid biosynthesis at the phytoene desaturase step (PDS);
 - g. inhibition of 4-hydroxyphenyl-pyruvate-dioxygenase (4-HPPD);
 - h. inhibition of carotenoid biosynthesis;
 - 5 i. inhibition of EPSP synthase;
 - j. inhibition of glutamine synthetase;
 - k. inhibition of DHP (dihydropteroate) synthase;
 - l. microtubule assembly inhibition;
 - m. inhibition of mitosis / microtubule organization;
 - 10 n. inhibition of cell division;
 - o. inhibition of VLCFAs;
 - p. inhibition of cell wall (cellulose) synthesis;
 - q. uncoupling (membrane disruption);
 - r. inhibition of lipid synthesis - not ACCase inhibition;
 - 15 s. action like indole acetic acid (synthetic auxins); and
 - t. inhibition of auxin transport;
8. The method of claim 1 wherein previously unknown metabolic states are identified as distinguished from known metabolic states associated with herbicide modes-of-action in an artificial neural network simulation.
- 20 9. The method of claim 1, wherein the biological samples are obtained from organisms of the same species.
- 25 10. The method of claim 1, wherein the sample is from a fungi tissue.
11. The method of claim 1, wherein the sample is from a yeast tissue.
12. The method of claim 1, wherein the sample is from a bacteria.
- 30 13. The method of claim 1, wherein the sample is from an animal tissue.
14. The method of claim 1, wherein the sample is from a plant tissue.

15. The method of claim 14, wherein said plant tissue is plant protoplast.
16. The method of claim 14, wherein said plant tissue is whole plant.
- 5 17. The method of claim 14, wherein said plant tissue is a partial plant.
18. The method of claim 14, wherein said plant tissue is callus tissue.
19. The method of claim 14, wherein said plant tissue is a cell suspension culture.
- 10 20. A method for determining the metabolic mode of action of a compound wherein said method comprises the method of claim 1 and said subject biological sample is from an organism treated with the compound, and said subject metabolic state indicates the metabolic mode of action of the compound.
- 15 21. A method for the determining the metabolic stress response in plants to stimuli wherein said method comprises the method of claim 1 and said subject biological sample is from an organism exposed to the stimuli, and said subject metabolic state indicates the metabolic stress response to the stimuli.
- 20 22. The method of claim 21, wherein the stimuli is a change in temperature, salinity or moisture.
23. A metabolic profiling process wherein said process comprises
- 25 a. growing organisms under controlled conditions;
- b. treating a control subset of the organisms with known bioregulators;
- c. treating a subject subset of the organisms with an uncharacterized bioregulator;
- d. preparing samples of tissues of the subsets of the organisms;
- 30 e. obtaining spectroscopic or chromatographic data of a plurality of metabolites from the samples;
- f. training an automated pattern recognition system by association of the spectroscopic or chromatographic data from the control subset of the

organisms treated with the known bioregulator to determine a control metabolic profile;

g. generating a mathematical model from the trained pattern recognition system based on spectroscopic or chromatographic data of the control subset of the organisms associated with the control metabolic profile;

h. applying the mathematical model to the spectroscopic or chromatographic data of the subject subset of the organisms to determine the subject metabolic profile; and,

i. comparing the subject metabolic profile to the control metabolic profile to determine the metabolic association of the uncharacterized bioregulator to the known bioregulator.

24. The method of claim 23, wherein the chromatographic technique is gas chromatography.
25. The method of claim 23, wherein the spectroscopic technique is nuclear magnetic resonance spectroscopy.
26. The method of claim 23, wherein the spectroscopic technique is mass spectroscopy.
27. The method of claim 23, wherein said method employs data obtained from both chromatographic and spectroscopic techniques.
28. The method of claim 23, wherein the pattern recognition analysis system comprises a neural network analysis.
29. The method of claim 23, wherein the metabolic profile results from a metabolic state selected from the group consisting of:
 - a. inhibition of acetyl CoA carboxylase (ACCase);
 - b. inhibition of acetolactate synthase (ALS) or acetohydroxyacid synthase (AHAS);
 - c. inhibition of photosynthesis at photosystem II;
 - d. photosystem-I-electron diversion;
 - e. inhibition of protoporphyrinogen oxidase (PPO);

- f. inhibition of carotenoid biosynthesis at the phytoene desaturase step (PDS);
- g. inhibition of 4-hydroxyphenyl-pyruvate-dioxygenase (4-HPPD);
- h. inhibition of carotenoid biosynthesis;
- 5 i. inhibition of EPSP synthase;
- j. inhibition of glutamine synthetase;
- k. inhibition of DHP (dihydropteroate) synthase;
- l. microtubule assembly inhibition;
- m. inhibition of mitosis / microtubule organization;
- 10 n. inhibition of cell division;
- o. inhibition of VLCFAs;
- p. inhibition of cell wall (cellulose) synthesis;
- q. uncoupling (membrane disruption);
- r. inhibition of lipid synthesis - not ACCase inhibition;
- 15 s. action like indole acetic acid (synthetic auxins); and
- t. inhibition of auxin transport.

30. The method of claim 23, wherein previously unknown metabolic profiles are identified as distinguished from known metabolic profiles associated with herbicide modes-
20 of-action in an artificial neural network simulation.

31. The method of claim 23, wherein the biological samples are obtained from organisms of the same species.

25 32. The method of claim 23, wherein the sample is from a fungi tissue.

33. The method of claim 23, wherein the sample is from a yeast tissue.

34. The method of claim 23, wherein the sample is from a bacteria.

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35. The method of claim 23, wherein the sample is from an animal tissue.

36. The method of claim 23, wherein the sample is from a plant tissue.

37. The method of claim 36, wherein said plant tissue is plant protoplast.
38. The method of claim 36, wherein said plant tissue is whole plant.
- 5 39. The method of claim 36, wherein said plant tissue is a partial plant.
40. The method of claim 36, wherein said plant tissue is callus tissue.
41. The method of claim 36, wherein said plant tissue is a cell suspension culture.
- 10 42. A metabolic profiling process wherein said process comprises
- a. growing organisms under controlled conditions;
 - b. selecting a control subset of the organisms with known phenotypic or genotypic traits;
 - 15 c. selecting a subject subset of the organisms with a potential unknown genetic modification or altered phenotype;
 - d. preparing samples of tissues of the subsets of the organisms;
 - e. obtaining spectroscopic or chromatographic data of a plurality of metabolites from the samples;
 - 20 f. training an automated pattern recognition system by association of the spectroscopic or chromatographic data from the control subset of the organisms to determine a control metabolic profile;
 - g. generating a mathematical model from the trained pattern recognition system based on spectroscopic or chromatographic data of the control subset
 - 25 of the organisms associated with the control metabolic profile;
 - h. applying the mathematical model to the spectroscopic or chromatographic data of the subject subset of the organisms to determine the subject metabolic profile; and,
 - i. comparing the subject metabolic profile to the control metabolic
 - 30 profile to determine the metabolic association of the potential unknown genetic modification or altered phenotype to the known phenotypic or genotypic traits.

43. The method of claim 42, wherein the chromatographic technique is gas chromatography.
44. The method of claim 42, wherein the spectroscopic technique is nuclear magnetic resonance spectroscopy.
45. The method of claim 42, wherein the spectroscopic technique is mass spectroscopy.
46. The method of claim 42, wherein said method employs data obtained from both chromatographic and spectroscopic techniques.
47. The method of claim 42, wherein the pattern recognition analysis system comprises a neural network analysis.
48. The method of claim 42, wherein the metabolic profile results from a metabolic state selected from the group consisting of:
- a. inhibition of acetyl CoA carboxylase (ACCase);
 - b. inhibition of acetolactate synthase (ALS) or acetohydroxyacid synthase (AHAS);
 - c. inhibition of photosynthesis at photosystem II;
 - d. photosystem-I-electron diversion;
 - e. inhibition of protoporphyrinogen oxidase (PPO);
 - f. inhibition of carotenoid biosynthesis at the phytoene desaturase step (PDS);
 - g. inhibition of 4-hydroxyphenyl-pyruvate-dioxygenase (4-HPPD);
 - h. inhibition of carotenoid biosynthesis;
 - i. inhibition of EPSP synthase;
 - j. inhibition of glutamine synthetase;
 - k. inhibition of DHP (dihydropteroate) synthase;
 - l. microtubule assembly inhibition;
 - m. inhibition of mitosis / microtubule organization;
 - n. inhibition of cell division;
 - o. inhibition of VLCFAs;
 - p. inhibition of cell wall (cellulose) synthesis;

- q. uncoupling (membrane disruption);
- r. inhibition of lipid synthesis - not ACCase inhibition;
- s. action like indole acetic acid (synthetic auxins); and
- t. inhibition of auxin transport.

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49. The method of claim 42, wherein previously unknown metabolic states are identified as distinguished from known metabolic states associated with herbicide modes-of-action in an artificial neural network simulation.

10 50. The method of claim 42, wherein the biological samples are obtained from organisms of the same species.

51. The method of claim 42, wherein the sample is from a fungi tissue.

15 52. The method of claim 42, wherein the sample is from a yeast tissue.

53. The method of claim 42, wherein the sample is from a bacteria.

54. The method of claim 42, wherein the sample is from an animal tissue.

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55. The method of claim 42, wherein the sample is from a plant tissue.

56. The method of claim 55, wherein said plant tissue is plant protoplast.

25 57. The method of claim 55, wherein said plant tissue is whole plant.

58. The method of claim 55, wherein said plant tissue is a partial plant.

59. The method of claim 55, wherein said plant tissue is callus tissue.

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60. The method of claim 55, wherein said plant tissue is a cell suspension culture.

61. A database of metabolic responses comprising data generated from the method of claim 1, claim 23 or claim 42.

62. The database of Claim 61 wherein the genetic alteration comprises a gene mutation.
63. The database of Claim 61 wherein the genetic alteration comprises a gene deletion.
- 5 64. The database of Claim 61 wherein the genetic alteration comprises a gene insertion.
65. The database of Claim 61 wherein the genetic alteration comprises gene activation change.
- 10 66. The database of Claim 65 where the gene activation change comprises a change in transcription factors.
67. The database of Claim 65 where the gene activation change comprises a change in promoters.
- 15 68. The database of Claim 61 wherein the genetic alteration comprises a genetic modification.
- 20 69. The database of Claim 68 wherein the genetic modification comprises knockout of gene activity.
70. The database of Claim 68 wherein the genetic modification comprises inactivation of gene activity.
- 25 71. The database of Claim 61 wherein the genetic alteration comprises insertion of genes.

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